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(71) Applicant: MALLINCKRODT MEDICAL, INC. [US/US]; 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

(72) Inventors: LYLE, Leon, R.; 1319 Webster Path Drive, Webster Groves, MO 63119 (US). THOMAS-MILLER, Beth; Apartment D, 8 Clermont Court, St. Louis, MO 63146 (US).

(74) Agents: McBRIDE, Thomas, P. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

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(54) Title: THERAPEUTIC TREATMENT FOR INHIBITING VASCULAR RESTENOSIS

(57) Abstract

A composition suitable for administration to a warm-blooded animal comprising an antisense oligonucleotide to the C-C chemokine family typified by MCP-1 and MIP-1-Alpha which may or may not be labeled with a radionuclide by means of a chelate ligand capable of administration to an animal to produce reliable visual imaging of areas of potential restenosis or to produce therapeutic effects on areas of potential restenosis.

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THERAPEUTIC TREATMENT FOR INHIBITING VASCULAR RESTENOSIS

FIELD OF THE INVENTION

This invention relates generally to novel compounds for therapeutic use, and more particularly, to specific molecularly interactive compounds, to methods of preparing and using such specific compounds, and to pharmaceutical compositions comprising these specific compounds for therapeutic use in areas of vascular injury, sites of inflammation, vascular atheromatous disease and/or restenosis.

BACKGROUND OF THE INVENTION

Balloon angioplasty, atherectomy, rotary ablation and similar therapeutic techniques used to improve circulation in vivo are finding ever-increasing application in therapeutic cardiology. Generally, balloon angioplasty procedures involve the introduction of a balloon-type catheter into the narrowed portion of an artery. The narrowing of the artery may be caused by different factors but most commonly is caused by a build-up of "atherosclerotic plaque". Once the catheter is positioned in the narrowed portion of the artery, the balloon portion of the catheter is inflated. The inflation of the balloon within the narrowed area of the artery serves to increase the diameter of the blood vessel thus improving circulation.

Often times, following a balloon angioplasty therapeutic procedure or similar therapeutic technique with attendant vascular injury, patients experience a re-narrowing or restenosis, of the artery within six months after having undergone the angioplasty therapeutic

treatment or after incurring the particular vascular injury. Restenosis is of considerable concern since its effects may be life threatening.

Therefore, the need for a suitable compound for therapeutic use to prevent restenosis following balloon angioplasty or similar therapeutic techniques which may cause vascular injury is of significant importance. It is an object of the present invention to meet this need.

SUMMARY OF THE INVENTION

The present invention discloses novel oligonucleotide, peptide, and polypeptide compounds, methods of preparing these compounds, pharmaceutical compositions comprising these compounds and the use of these compounds in balloon-type catheters for therapeutic treatment to inhibit vascular restenosis. Restenosis is a recurrent stenosis, i.e., a narrowing or stricture of a duct or canal. Restenosis and the development of atheromatous lesions (the reason for the procedure in the first place) share several common pathological elements such as the accumulation of monocytes and macrophages at the area of injury or inflammation and the proliferation of vascular smooth muscle. Growth factors which induce this proliferation of vascular smooth muscle and thus cause restenosis, arise in large part from the monocytes and macrophages which infiltrate the injured area in response to inflammatory stimuli. The monocytes and macrophages present in the tissue represent stages of differentiation of the same cell lineage. The cells are referred to as monocytes when in the blood. Upon deposition in tissue, the cells are called macrophages.

Monocyte Chemotactic Protein-1, hereinafter

referred to as "MCP-1" is a member of the "C-C" family of chemo attractant cytokines or "chemokines". It is a potent stimulator of monocyte chemotaxis and has an extremely high degree of specificity for this cell type. Other family members include Human Macrophage Inflammatory Protein-1 (HuMIP-1) Alpha and Beta, Monocyte Chemotactic Protein-2 (MCP-2), RANTES, RANTES precursor and I-309. All of these chemokines incorporate a cysteine-cysteine (C-C) motif, but MCP-1 and MIP-1 Alpha are the ones most highly specific for monocytes and macrophages. MCP-1 and MIP-1 Alpha as well as the rest of the C-C chemokine family are produced by injured vascular smooth muscle cells. The C-C chemokines, e.g., MCP-1 so produced attract the monocytes and macrophages which infiltrate the area releasing growth factors and resulting in proliferation of vascular smooth muscle and restenosis.

In using a molecularly interactive therapeutic compound to inhibit vascular restenosis as discussed herein, the compound must be highly specific. High specificity, which is essential in such therapeutic compounds, means that the compound, after having been introduced into the body, is active to a greater degree against the target molecule or tissue, i.e. the area of possible restenosis, than on other non-target molecules or tissues. In using oligonucleotides or peptides or polypeptides as therapeutic compounds, the high specificity of the particular agent used provides for the strong accumulation or retention of the therapeutic compound to the target molecule or the specific tissue or tissues targeted. In the case of the present invention, the site of accumulation and retention is in areas of injured vascular smooth muscle cells as compared with the accumulation and retention concentration thereof in other non-target tissues.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention, a balloon-type catheter such as a balloon infusion catheter is coated or filled with a total, partial or synthetic antisense oligonucleotide or peptide to monocyte chemoattractant protein (MCP) material, such as monocyte chemoattractant protein-1 (MCP-1), MIP-1 Alpha or other members of the C-C family of chemotactic cytokines or chemokines hereinafter referred to as "antisense MCP-1" or like member of the C-C family of chemokines as mentioned above and described in more detail below. However, for means of simplicity MCP-1 will be used as an example throughout although any other chemokine family member such as MIP-1 Alpha would also be a suitable target.

An antisense oligonucleotide such as an antisense oligonucleotide to MCP-1 inhibits the translation or transcription of MCP-1 mRNA within the vascular smooth muscle cells or surrounding interstitial space. Accordingly, MCP-1 production is severely inhibited. In the absence of MCP-1, monocytes are not attracted to the area of vascular injury in their usual numbers. As a result of the monocytes not infiltrating the area, growth factors (GFs) are not released. The relative lack of GFs does not support the proliferation of vascular smooth muscle cells which cause restenosis in cases of vascular injury. This is likewise true in the case of antisense oligonucleotide constructs to MIP-1 Alpha, MIP-1 Beta, RANTES, RANTES precursor, and I-309. It may be beneficial to administer two or more different antisense oligonucleotides or derivatives thereof simultaneously to inhibit production of two or more cytokines.

Therapeutic treatment of vascular restenosis can also be achieved and augmented through the use of another embodiment of the present invention whereby the antisense oligonucleotide, to members of the C-C chemokine family, e.g., MCP-1 is labelled with a radionuclide for therapeutic use. Radiolabelled antisense MCP-1 compounds for therapeutic use may be constructed using high energy Alpha or Beta emitting isotopes rather than the pure gamma emitters customarily used for diagnostic purposes which is also possible and will be discussed in more detail below.

Mature members of the C-C chemokine family are produced by post-translational modification of larger peptides. The sense sequence of the mature MCP-1 polypeptide is as follows:

NH_2-M G X P D A I N A P V T C C Y N F T N R K I
S V Q R L A S Y R R I T S S K C P K E A V I F K
T I V A K E I C A D P K Q K W V Q D S M D H L D
K Q T Q T P K T-COOH;

wherein A in each of the examples, represents Alanine, B represents Asparagine or Aspartic Acid, C represents Cysteine, D represents Aspartic Acid, E represents Glutamic Acid, F represents Phenylalanine, G represents Glycine, H represents Histidine, I represents Isoleucine, K represents Lysine, L represents Leucine, M represents Methionine, N represents Asparagine, P represents Proline, Q represents Glutamine, R represents Arginine, S represents Serine, T represents Threonine, V represents Valine, W represents Tryptophan, X represents an unspecified or variable amino acid, Y represents Tyrosine and Z represents Glutamine Acid.

The oligonucleotides in the messenger ribonucleic acid (mRNA), antisense deoxyribonucleic acid (DNA) and antisense RNA corresponding to mRNA sequences for MCP-1 are as follows.

mRNA:

5′ -AUG CAG CCA GAU GCA AUC AAU GCC CCA GUC ACC UGC UGU UAU AAC UUC ACC AAU AGG AAG AUC UCA GUG CAG AGG CUC GCG AGC UAU AGA AGA AUC ACC AGC AGC AAG UGU CCC AAA GAA GCU GUG AUC UUC AAG ACC AUU GUG GCC AAG GAG AUG UGU GCU GAC CCC AAG CAG AAG UGG GUU CAG GAU UCC AUG GAC CAC CUG GAC AAG CAA ACC CAA ACU CCG AAG ACU - 3′;

Antisense DNA:

5′ -TAC GTC GGT CTA CGT TAG TTA CGG GGT CAG TGG ACG ACA ATA TTG AAG TGG TTA TCC TTC TAG AGT CAC GTC TCC GAG CGC TCG ATA TCT TCT TAG TGG TCG TGG TTC ACA GGG TTT CTT CGA CAC TAG AAG TTC TGG TAA CAC GGG TTC CTC TAG ACA CGA CTG GGG TTC GTC TTC ACC CAA GTC GTA AGG TAC CTG GTG GAC CTG TTC GTT TGG GTT TGA GGC TTC TGA - 3′; and

Antisense RNA:

5′ -UAC GUC GGU CUA CGU UAG UUA CGG GGU CAG UGG ACG ACA AUA UUG AAG UGG UUA UCC UUC UAG AGU CAC GUC UCC GAG CGC UCG AUA UCU UCU UAG UGG UCG UCG UUC ACA GGG UUU CUU CGA CAC UAG AAG UUC UGG UAA CAC CGG UUC CUC UAG ACA CGA CUG GGG UUC GUC UUC ACC CAA GUC CUA AGG UAC CUG GUG GAC CUG UUC GUU UGG GUU UGA GGC UUC UGA - 3′;

wherein A=Adenine, T=Thymine, C=Cytosine, G=Guanine, U=Uracil, B=not A, D=not C, F=not G, K=G or T, M=A or C,

N=A, C, G or T, R = A or G, S=C or G, V=not T, W=A or T and Y=C or T.

In targeting antisense oligonucleotides into smooth muscle cells it is not necessary that the entire oligonucleotide sequence for the mature peptide be present. Effective complementary binding may reside in a smaller portion of the molecule. Short segments of antisense oligonucleotides may be prepared to the mRNA to effectively block the translation of the mature peptide. The C-C motif, from which this group of chemokines derives their name, is a very important structural feature which confers structural integrity upon the molecule. It is therefore best to target this area for inhibition of the synthesis of this class of molecules. For example, peptide sequences adjacent to the C-C structural motif for members of the "C-C" family of chemokines are very effective targets and are listed below.

The sense MCP-1 polypeptide structural motif is as follows when flanked with five residues on either side as referenced in Yashimura, T., et al., FEBS Letters, vol. 244; pp. 487-493 (1989):

NH_2 -N A P V T C C Y N F T R -COOH;

Antisense RNA:

5′ -UUA CGG GGU CAG UGG ACG ACA AUA UUG AAG UGG UUA - 3′; and

Antisense DNA:

5′ -TTA CGG GGT CAG TGG ACE ACA ATA TTG AAG TGG TTA - 3′.

The sense MIP-1 Alpha polypeptide structural motif sequence is as follows when flanked with five residues on either side as referenced in Blum, S., et al., DNA and Cell Biology, Vol. 9; pp. 589-602 (1990):

NH_2 -D T P T A C C F S Y T S -COOH;

MIP -1 Alpha Antisense RNA:

5′ -CUG UGC GGC UGG CGG ACG ACG AAG UCG AUG UGG AGG - 3′; and

MIP -1 Alpha Antisense DNA:

5′ -CTG TGC GGC TGG CGG ACG ACG AAG TCG ATG TGG AGG - 3′.

The sense MIP-1 Beta polypeptide structural motif sequence is as follows when flanked with five residues on either side:

NH_2 -D P P T S C C F S Y T S -COOH

Antisense RNA:

5′ -CUR GGN GGN UGN WSN ACR ACR AAR WSN AUR UGN WSN - 3′; and

Antisense DNA:

5′ -CTR GGN GGN TGN WSN ACR ACR AAR WSN ATR TGN WSN - 3′;

wherein R=A or G; N = A, C, G or T/U. W = A or T; S = C or G.

The sense RANTES and RANTES precursor polypeptide structural motif sequence is as follows when flanked with five residues on either side as referenced in Schall, T.S., et al., Journal of Immunology, Vol. 141; pp. 1018-1025, (1988):

NH_2 -S D T T P C C F A Y I A -COOH;

Antisense RNA:

5′ -AGC CUG UGG UGU GGG ACG ACG AAA CGG AUG UAA CGG - 3′; and

Antisense DNA:

5′ -AGC CTG TGG TGT GGG ACG ACG AAA CGG ATG TAA CGG - 3′.

The sense I-309 polypeptide structural motif sequence is as follows when flanked with five residues on either side as referenced in Miller, M.D., et al., Journal of Immunology, Vol. 145; pp. 2737-2744 (1990):

NH_2 -V P F S R C C F S F A E -COOH;

Antisense RNA:

5′ -CAU GGG AAG AGG UCU ACA ACG AAG AGU AAA CGC CUC - 3′; and

Antisense DNA:

5′ -CAT GGG AAG AGG TCT ACA ACG AAG AGT AAA CGC CTC - 3′.

It may also be useful to replace some oxygen atoms in the phosphate backbone with thiol groups to inhibit degradation in vivo.

In the present invention, the antisense MCP-1 oligonucleotide to a molecule of the C-C chemokine family having similar specificity, may be administered in vivo using a balloon infusion catheter with holes in it for delivery to the particular target site to prevent life-threatening restenosis. The antisense MCP-1 oligonucleotide may also be radiolabeled prior to administration, using more than one method. The objective in radiolabeling is to increase therapeutic effect by

bringing this cytostatic properly to bear upon smooth muscle and to force the cells into apoptosis.

Still another embodiment of the present invention is the introduction of an antisense oligonucleotide or the gene for the synthesis of antisense MCP-1 oligonucleotide into individual vascular smooth muscle cells in area(s) of vascular injury.

When introducing a gene for the production of an antisense MCP-1 oligonucleotide into the vascular smooth muscle cells, replication of the antisense MCP-1 is aided by placing it under the control of a tissue specific promoter such as the smooth muscle Alpha actin promoter to prevent life-threatening vascular restenosis. Viral promoters may also be used such as the cytomegalovirus (CMV) promoter.

Such introduction is affected by infusion with a high concentration of oligonucleotide into the smooth muscle tissues with a balloon infusion catheter. This typically requires high pressure(s) (greater than 2 atmospheres) and high concentrations of oligonucleotides (greater than 12.5 micrograms per milliliter) and is aided by agents which help to increase the solubility of membranes such as lipid rich liposomes.

If based on antisense or DNA or RNA so as to bind to MCP-1 mRNA and prevent translation, the sequence to be introduced is derived from the antisense or DNA or RNA sequences previously given on pages 5 through 8.

It is important to note that effective inhibition of translation need not require the entire sequence. Appropriate specificity and ability to inhibit may be

conferred with a sequence of approximately 15 to 30 nucleotides.

As noted above, the cysteine cysteine (C-C) motif is a common feature characteristic of this family of chemokines and maintenance of this motif is a critical factor in preservation of biological activity. Therefore nucleotide sequences which would inhibit cysteine cysteine (C-C) translation with preservation of specificity are particularly effective. For example the sense mRNA region 5'- AAU GCC CCA GUC ACC UGC UGU UAU AAC UUC ACC AAU -3', or the antisense RNA construct 5'- UUA CGG GGU CAG UGG ACG ACA AUA UUG AAG UGG UUA-3' which would target the MCP-1 mRNA sequence that stipulates the peptide shown on page 6.

In a further embodiment of this invention, an antisense oligonucleotide was designed to inhibit translations of both the MCP-1 and MIP-1 Alpha Chemokine messages. The designed antisense oligonucleotide sequence is as follows:

5' -ACA CGA CUG GGG UUC CUC UUC ACC CAA GUC -3'.

This antisense oligonucleotide was designed by first examining the amino acid sequences of MCP-1 and MIP-1 Alpha for regions of homology. By using the computer program MacVector, a high degree of homology was observed between residues 53 through 62 of MCP-1 and 55 through 64 in MIP-1 Alpha. A stretch of 10 residues was chosen so that the corresponding RNA would consist of 30 bases.

The DNA that codes for both MCP-1 and MIP-1 Alpha has been cloned and reported in the literature. Using the information, one antisense oligonucleotide that will bind to the mRNA's coding for both MCP-1 and MIP-1 Alpha was

designed. The above antisense oligonucleotide contains only one mismatch with the mRNA for MCP-1 occuring at base 16. C was substituted for G because this purine would not be able to base-pair with the G at position 16 of mRNA for MIP-1 Alpha because of steric problems. Three mismatches between the designed antisense oligonucleotide and the mRNA for MIP-1 Alpha exist. However, some base-pairing should still occur at these sites because none of the interactions include two purines, which would cause steric problems.

In a further embodiment of this invention, therapeutic effects of antisense oligonucleotides upon potentially proliferating smooth muscle cells are achieved by radiolabelling the antisense MCP-1 oligonucleotide with a suitable isotope such phosphorous 32 or phosphorous 33.

Antisense peptides

An antisense peptide is specified by the DNA strand complementary to that which specifies the ordinary sense peptide. These antisense peptides function by "hydropathic complementarity" to give binding activity with its corresponding sense peptides and can function as receptor like molecules in affinity chromatography as explained by Souza, S.J.U. and Bretani, R. J., Biol. Chem. 267: 13763-13773 (1992). When an antisense peptide is used, one obtains complementary binding to and inactivation of the mature MCP-1 polypeptide.

The antisense MCP-1 of the present invention is represented by the following sequence:

NH_2 -X G L R X L R G X X T T X L K X L X F X X X
V X X R X X X X X X X X F T G F L R X X K F X X
X R F L X T R L G F V F T X V L X Y L V X L F V

X V X G F X-COOH;

In targeting mature C-C cytokine family, e.g., MCP-1 polypeptide with antisense MCP-1 polypeptide, it is not necessary that the complete seventy-six (76) residue sequence be present. Effective complementary binding may reside in a smaller portion of the molecule. Through substitution in the antisense MCP-1 polypeptide sequence, and perhaps incorporating (d) amino acid enantiomorphs, retroinverse bonds peptidomimetics and the like, additional useful peptides are developed without affecting complementary binding specificity and affinity desired.

The reaction in radiolabelling antisense peptides generally takes place between the amino groups in the peptide and the carbonyl group in the active ester of a specific ligand to form an amide bond. In particular, the peptides can be radiolabelled using either a conventional method referred to as "post-formed chelate approach" or by a recent method referred to as "pre-formed chelate approach" developed by Fritzberg et al., U.S. Patent Numbers 4,965,392 and 5,037,630 incorporated herein by reference. In the "pre-formed approach," the desired ligand is complexed with the radionuclide and then conjugated to antisense MCP-1 polypeptide or a molecule having antisense MCP-1 activity. In the "post-formed approach," the desired ligand is first conjugated to the antisense peptide and the resulting conjugate is incubated with the radionuclide along with a reducing agent. In the present invention, the latter approach has the additional advantage of allowing preparation of the complex in kit form. Users merely add the radionuclide to the ligand antisense MCP-1 conjugate or a derivative thereof for labelling to occur.

It is important to note an unique mechanism of the present invention whereby the conjugation reaction will only occur when the Alpha amino group is in the "free base" form, i.e., deprotonated to the NH_2 form. If the amino group is protonated, i.e., in the NH_3^+ form, the reaction will not occur. Therefore, in the molecules of the present invention it is potentially important to perform the conjugation at neutral pH or within the range of 7.0 to 9.5 to avoid deprotonation of any epsilon-amino groups of lysine, or K. Avoiding the deprotonation of epsilon-amino groups involved in binding prevents the formation of a chelate complex which may interfere with the ability of the antisense peptide to form a complementary complex with MCP-1. In the present invention, binding preferably occurs on the Alpha amino group in order to avoid potential interference with the ability of the antisense MCP-1 peptide to form a complementary complex with sense.

Using either method of labelling antisense C-C chemokines, e.g., MCP-1, any suitable ligand can be used to incorporate the preferred radionuclide metal ion such as for example but not limited to technetium, rhenium, indium, gallium, samarium, holmium, yttrium, copper, or cobalt, and more particularly, yttrium-90, rhenium-188, rhenium-186, indium-111, technetium-99m, and derivatives thereof. The choice of the ligand entirely depends on the type of metal ion desired for therapeutic or even diagnostic purposes. For example, if the radionuclide is a transition element such as technetium or rhenium, then ligands containing amine, amide, and thiols are preferred to form a stable complex whereas if the radionuclide is a lanthanide element, then polyaminocarboxylates or phenolate type ligands are preferable.

The above-described unique characteristics of the

present invention make the radiolabelled antisense MCP-1 polypeptide and its derivatives very attractive for therapeutic purposes or even diagnostic uses to identify sites of restenosis and/or vascular injury. The compounds of the present invention may be labelled with any radionuclide favorable for these purposes. Such suitable radionuclides for radiotherapy include but are not limited to rhenium-186, copper-67, rhenium-188 and cobalt-60. For diagnostic purposes the most suitable radionuclides include but are not limited to the transition metals as exemplified by technetium-99m and copper-62.

Due to the unique mechanism employed in the present invention to label the Alpha amino group of antisense MCP-1 peptide and avoid the epsilon amino group(s) (which could inhibit the ability of antisense MCP-1 peptides to bind to its complementary sense strand) a significantly advantageous radiolabelled peptide compound for radiotherapy and diagnostic imaging of areas of potential restenosis is achieved.

As previously noted, a preferred embodiment of the present invention is the antisense peptide, polypeptide or protein to MCP-1 or derivatives thereof used alone to prevent vascular restenosis. However, additional embodiments of the present invention include antisense MCP-1 or derivatives thereof radiolabelled using a pre-formed or post-formed methodology.

In a preferred embodiment according to the present invention, an antisense C-C cytokine, e.g., MCP-1 or a molecule having sense MCP-1 interactive capability is first bonded to the N_3S aminothiol ligand which is illustrated in Figure 1

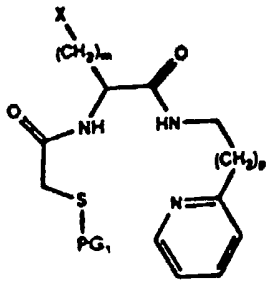

Figure 1

wherein m is a whole number less than eleven and preferably 3; p is either 0 or 1; PG_1 is a suitable sulfur protecting group selected from the group consisting of C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted benzoyl -whereby alkanoyl is preferable, C_{1-20} S-acyl groups such as benzyl, t-butyl, trityl, 4-methoxybenzyl and 2,4-dimethoxybenzyl -whereby 2,4-dimethoxybenzyl is preferable, C_{1-10} alkoxyalkyl such as methoxymethyl, ethoxyethyl and tetrahydropyranyl -whereby tetrahydropyranyl is preferable, carbamoyl, and C_{1-10} alkoxycarbonyl such as t-butoxycarbonyl and methoxycarbonyl -whereby t-butoxycarbonyl is preferable; and X is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, maleimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl -whereby N-methoxylcabamoyl is preferable.

In another preferred embodiment according to the present invention, antisense MCP-1 or a molecule having sense MCP-1 interactive capability is bonded to the N_2S_2 aminothiol ligand which is illustrated in Figure 2;

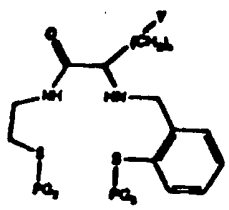

Figure 2

wherein n is a whole number less than eleven and preferably 3; PG_2 and PG_3 may be the same or different sulfur protecting groups selected from the group consisting of C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted-benzoyl -whereby alkanoyl is preferable, C_{1-20} alkyl groups such as benzyl, t-butyl, 4-methoxybenzyl, trityl and 2,4-dimethoxybenzyl -whereby 2,4-dimethoxybenzyl is preferable, C_{1-10} alkoxyalkyl such as for example methoxymethyl, ethoxyethyl, and tetrahydropyranyl -whereby tetrahydropyranyl is preferable, carbamoyl and C_{1-10} alkoxycarbonyl such as methoxycarbonyl, ethoxycarbonyl and t-butoxycarbonyl -whereby t-butoxycarbonyl is preferable; and Y is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, maleimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl, and C_{1-10} N-alkoxycarbamoyl -whereby N-methoxylcabamoyl is preferable.

In another preferred embodiment of the present invention, an antisense C-C cytokine, e.g., to MCP-1 or a molecule having interactive capability with sense MCP-1 is conjugated with the ligand illustrated in Figure 3,

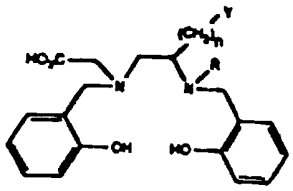

Figure 3

wherein n varies from 1 to 10, and Y is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothioganate, imidate, maleimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl,

haloacetyl, and C_{1-10} N-alkoxycarbamoyl such as N-methoxycarbamoyl and t-butoxycarbamonyl -whereby t-butoxycarbamonyl is preferable; and R is selected from the group consisting of hydrogen and C_{1-10} alkyl such as methyl and t-butyl -whereby t-butyl is preferable.

In another preferred embodiment, an antisense C-C chemokine, e.g., MCP-1 or a molecule having interactive capability with sense MCP-1 can be conjugated with the metal complex illustrated in Figure 4

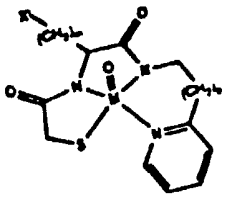

Figure 4

wherein m is a whole number less than eleven and more preferably 3; p is either 0 or 1; X' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, maleimide, chlorocarbonyl, chlorosulfonyl, succininimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl such as N-methoxycarbamoyl and t-butoxycarbamoyl -whereby t-butoxycarbamoyl is preferable and M is a radionuclide suitable for diagnostic imaging or therapeutic use such as technetium, rhenium, copper, cobalt, indium, gallium, samarium, yttrium and holmium.

In another preferred embodiment, an antisense C-C chemokine, e.g., MCP-1 or a molecule having interactive capability with sense MCP-1 can be conjugated with a metal complex as illustrated in Figure 5 wherein Y' and n are defined the same respectively as Y and n in Figure 3 and M is defined the same as M in Figure 4.

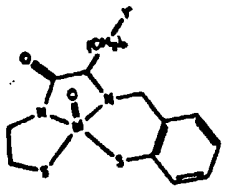

Figure 5

In another preferred embodiment, an antisense C-C chemokine, e.g., MCP-1 or a molecule having interactive capability with sense MCP-1 can be conjugated with a metal complex as shown in Figure 6.

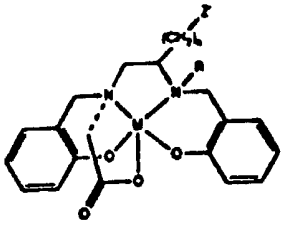

Figure 6

wherein Z', q and R are defined the same respectively as Y, n and R of Figure 3 and M is defined the same as M in Figure 4.

In another preferred embodiment, an antisense C-C chemokine, e.g., MCP-1 or a molecule having interactive capability with sense MCP-1 can be conjugated with a metal complex as shown in Figure 7.

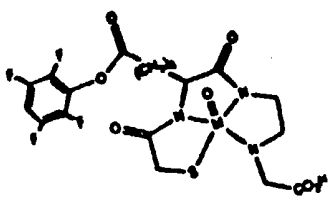

Figure 7

wherein M is defined the same as M in Figure 4.

Common esters which have been found useful in this labelling technique are o- and p- nitrophenyl, 2-chloro-4-nitrophenyl, cyanomethyl, 2-mercaptopyridyl, hydroxybenztriazole, N-hydroxysuccinimide, trichlorophenyl, tetrafluorophenyl, thiophenyl, tetrafluorothiophenyl, o-nitro-p-sulfophenyl, N-hydroxyphthalimide and the like. For the most part, the esters will be formed from the reaction of the carboxylate with an activated phenol, particularly, nitro-activated phenols, or a cyclic compound based on hydroxylamine.

The advantages of using sulfur protecting groups include the fact that a separate step for removal of the sulfur-protective group is not necessary. The protecting groups are displaced from the compound during the labelling in what is believed to be a metal-assisted acid cleavage: i.e., the protective groups are displaced in the presence of a radionuclide at an acid pH and the radionuclide is bound by the chelating compound. The radiolabeling procedure thus is simplified, which is a significant advantage when the chelating compounds are to be radiolabelled in a hospital laboratory shortly before use. Additionally, another advantage of the present invention is that the basic pH conditions and harsh conditions associated with certain known radiolabeling procedures or procedures for removal of other sulfur protected groups are avoided. Thus, base-sensitive groups on the chelating compounds survive the radio-labelling step intact. Suitable sulfur-protecting groups, when taken together with the sulfur atom to be protected, include hemithioacetal groups such as ethoxyethyl, tetrahydrofuranyl,

methoxymethyl, and tetrahydropyranyl. Other suitable sulfur protecting groups are C_{1-20} acyl groups, preferably alkanoyl or benzoyl. Other possible formulas for the chelating compounds are described in U.S. Patent Number 4,965,392 incorporated herein by reference.

Synthesis of the radionuclide bifunctional chelate and subsequent conjugation to antisense MCP-1, or a derivative thereof, can be performed as described in U.S. Patent Number 4,965,392 incorporated herein by reference and related technologies as covered by U.S. patent numbers 4,837,003, 4,732,974 and 4,659,839, each incorporated herein by reference.

After purification, the radiolabelled antisense C-C chemokine, e.g., MCP-1, or derivatives thereof, may be injected into a patient for therapeutic use or even diagnostic imaging depending on the radionuclide used. The radiolabelled antisense MCP-1 compound of the present invention is capable of radiotherapeutic use or reliably visualizing areas of potential restenosis within minutes post-injection. The antisense MCP-1 peptide when radiolabelled with the Re-186 or Re-188 triamide thiolate bifunctional chelate is particularly efficacious as an in vivo radiotherapeutic agent for areas of restenosis.

Each of the embodiments of the present invention are described in still greater detail in the illustrative examples which follow:

Example 1:

Antisense RNA or DNA or a derivative thereof for purposes of inhibition of translation is prepared by oligonucleotide synthesis using the solid phase phosphotrizster method detailed by Woods, et al., Proc. Natl. Acad. Sci. USA, Vol. 79; pp. 5661-5665 (1982) and

suspended to a concentration of between 10 and 500 micrograms per milliliter in 10mM Tris chloride with 1mM ethylenediaminetetraacetic acid (EDTA) and infused into the lesion using a balloon infusion catheter at pressures of two to eight atmospheres. Contact time should be in the range of 5 to 30 minutes. If it is desired to radiolabel the preparation with phosphorus -32 or phosphorus-33 to increase therapeutic effect, phosphorus-32 or phosphorus-33 labeled nucleotides are prepared using the methods given by Maxam, A.M. and Gilbert, W., Pro. Natl. Acad. Sci. USA, Vol. 75; pp. 560-564 (1977).

Example 2:

A solution of antisense MCP-1 peptide, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand illustrated in Figure 1 (wherein m=2, p=1, PG_1 is benzoyl, and X is succinimidyloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired antisense MCP-1 conjugate.

Example 3:

A solution of antisense MCP-1 peptide, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand illustrated in Figure 2 (wherein n=2, PG_2 and PG_3 are benzoyl, and Y is succinimidyloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the

solution is lyophilized to give the desired antisense MCP-1 conjugate.

Example 4:

A solution of antisense MCP-1 peptide, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand illustrated in Figure 3 (wherein q=4, and Z is succinimidyloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired antisense MCP-1 conjugate.

Example 5:

To 100 uL of a solution containing 5 mg of sodium gluconate and 0.1 mg of stannous chloride in water, 500 ul of 99m-Tc04 (pertechnetate) is added. After incubation at room temperature for about 10 minutes, a solution of 500 uL of the antisense MCP-1 polypeptide, or derivatives thereof, conjugates (1 mg/mL in 0.1 M carbonate/bicarbonate buffer, pH 9.5) as described in Examples 1 or 2 is then added and the entire mixture is incubated at 37°C for about 1 hour. The desired labelled peptide is separated from unreacted 99mTc-gluconate and other small molecular weight impurities by gel filtration chromatography (Sephadex G-50) using phosphine buffered physiological saline, (hereinafter PBS), 0.15M NaCl, pH 7.4 as eluent.

Example 6:

A mixture of gentisic acid (25 mg), inositol (10 mg), and the antisense MCP-1 polypeptide, or derivatives thereof, conjugate (500 uL, 1 mg/mL in water) was treated with In-111 indium chloride in 0.05 M HCl. The solution

was allowed to incubate at room temperature for about 30 minutes. The desired labelled peptide is separated from unreacted In-111 indium salts and other small molecular weight impurities by gel filtration chromatography (Sephadex G-50) using phosphine buffered physiological saline, (PBS), 0.15M NaCl as eluent.

Example 7:

Antisense DNA or a derivative thereof for purposes of inhibition of MCP-1 synthesis by inhibition of transcription by self replication within smooth muscle cells is prepared by introduction of such DNA sequences into a plasmid (a circular piece of DNA) consisting of a smooth muscle actin or viral promoter coupled to antisense DNA to MCP-1 and appropriate start and stop signals. This plasmid is introduced into smooth muscle cells by using a balloon infusion catheter. The plasmid DNA is suspended to a concentration of between 10 and 100 micrograms per milliliter in Tris chloride EDTA (10 mM, 1 mM ETDA) (TE) and is infused at a pressure of between 2 and 8 atmospheres. Infusion time varies between 5 and 30 minutes.

After the antisense MCP-1 polypeptide, oligonucleotide or a derivative thereof is prepared and optionally labelled according to the procedure described above, the compound is used with a pharmaceutically acceptable carrier in a method of performing therapy or radiotherapy or a method of performing a diagnostic imaging procedure using a gamma camera or like device. These procedures involve injecting or administering, for example by means of a balloon injector catheter, to a warm-blooded animal an effective amount of the present invention and then in the case of diagnostic use, exposing the warm-blooded animal to an imaging procedure using a suitable

detector, e.g. a gamma camera. Images are obtained by recording emitted radiation of tissue or the pathological process in which the radioactive peptide or oligonucleotide has been incorporated, which in the present case are potential sites of restenosis, thereby imaging at least a portion of the body of the warm-blooded animal. Pharmaceutically acceptable carriers for either diagnostic or therapeutic use include those that are suitable for injection or administration such as aqueous buffer solutions, e.g. tris (hydroxymethyl)aminomethane (and its salts), chloride phosphate, citrate, bicarbonate, etc., sterile water for injection, physiological saline, and balanced ionic solutions containing chloride and/or bicarbonate salts of normal blood plasma cations such as Ca^{2+}, Na^{+}, K^{+} and Mg^{2+}. Other buffer solutions are described in Remington's Practice of Pharmacy, 11th edition, for example on page 170. The carriers may contain a chelating agent, e.g. a small amount of ethylenediaminetetraacetic acid (EDTA), calcium, disodium salt, or other pharmaceutically acceptable chelating agents.

The concentration of the labelled or unlabelled peptide and the pharmaceutically acceptable carrier, for example in an aqueous medium, varies with the particular field of use. A sufficient amount is present in the pharmaceutically acceptable carrier in the present invention when satisfactory visualization of areas of vascular injury is achievable or satisfactory therapeutic results are achievable.

The composition is administered to the warm-blooded animals so that the composition remains in the living animal for about six to seven hours, although shorter and longer residence periods are normally acceptable.

The antisense MCP-1 compounds of the present invention or antisense MCP-1 derivative thereof, prepared as described herein, provide means of in vivo therapeutic, radiotherapeutic or diagnostic imaging of areas of potential restenosis.

After consideration of the above specification, it will be appreciated that many improvements and modifications in the details may be made without departing from the spirit and scope of the invention. It is to be understood, therefore, that the invention is in no way limited, except as defined by the appended claims.

We claim:

1. The oligonucleotide defined by antisense sequence 5'-ACA CGA CUG GGG UUC CUC UUC ACC CAA GUC-3'.

2. A composition suitable for administration to a warm-blooded animal comprising said oligonucleotide of claim 1 or a derivative thereof to inhibit translation of mRNA for members of a C-C chemokine family typified by MCP-1 and MIP-1 Alpha, so as to inhibit vascular restenosis.

3. A method of in vivo vascular therapy, comprising administering to a warm-blooded animal a therapeutically effective amount of the oligonucleotide of claim 1 or a derivative thereof to inhibit translation of mRNA for members of C-C chemokine family typified by MCP-1 and MIP-1 Alpha so as to inhibit vascular restenosis.

4. The oligonucleotide of claim 1 or a derivative thereof capable of inhibiting vascular restenosis upon in vivo administration.

5. The oligonucleotide of claim 1, 2, 3 or 4 wherein said oligonucleotide is labeled with Phosphorus -32 or Phosphorus -33 and is capable of administration to a warm-blooded animal to inhibit vascular restenosis.

6. A plasmid construct consisting of the oligonucleotide of claim 1 or a derivative thereof linked to a smooth muscle actin or viral promoter capable of replication within smooth muscle cells to produce therapeutic effects on restenosis.

7. A composition suitable for administration to a warm-blooded animal comprising the oligonucleotide of claim 1,

antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof to inhibit vascular restenosis.

8. A method of in vivo vascular therapy, comprising administering to a warm-blooded animal a therapeutically-effective amount of the oligonucleotide of claim 1 an antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof to inhibit vascular restenosis.

9. The oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof capable of inhibiting vascular restenosis upon in vivo administration.

10. The oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof, wherein said oligonucleotide or peptide is labeled with a radionuclide by means of a chelate capable of administration to a warm-blooded animal to inhibit vascular restenosis.

11. A therapeutic composition suitable for administration to a warm-blooded animal comprising said oligonucleotide of claim 1 labeled with Re-186 or Re-188 by means of a triamide thiolate (N_3S) chelate capable of administration to an animal to produce therapeutic effects on areas of restenosis.

12. A method of performing a therapeutic procedure, which

comprises administering to a warm-blooded animal a therapeutically-effective amount of said oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof labeled with Re-186 or Re-188 by means of a triamide thiolate (N_3S) chelate to allow for therapeutic effects on areas of restenosis.

13. The oligonucleotide of claim 1 labeled with Re-186 or Re-188 by means of a triamide thiolate (N_3S) chelate.

14. The oligonucleotide of claim 1 of claim 13 wherein said antisense oligonucleotide labeled with Re-186 or Re-188 Re by means of a triamide thiolate (N_3S) chelate is capable of administration to a warm-blooded animal to produce therapeutic effects on areas of restenosis post-administration.

15. A diagnostic composition suitable for administration to a warm-blooded animal comprising said oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivative thereof labeled with a suitable radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate capable of administrationto an animal to produce reliable diagnostic imaging of areas of potential restenosis.

16. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of said oligonucliotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof labeled with a suitable radionuclide by means of a triamide thiolate (N_3S) or

diamide dithiolate (N_2S_2) chelate to allow for diagnostic imaging of areas of potential restenosis.

17. The oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivative thereof labeled with a suitable radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate.

18. The oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof, wherein said oligonucleotides or peptides are labeled with a suitable radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of areas of potential restenosis within two and one half hours post-injection.

19. A therapeutic composition suitable for administration to a warm-blooded animal comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof labeled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a suitable radioactive isotope capable of administration to an animal to produce therapeutic effects on areas of potential restenosis.

20. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal a therapeutically-effective amount of the oligonucleotide of claim 1 antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivative thereof labeled with a triamide

thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a suitable radioactive isotope to produce therapeutic effects on areas of potential restenosis.

21. The oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 peptide or a combination of two or more thereof or derivatives thereof labeled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a radioactive isotope.

22. A composition suitable for administration to a warm-blooded animal comprising an antisense oligonucleotide with MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES or I-309 interactive capability capable of administration to an animal to produce therapeutic effects on areas of potential restenosis.

23. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal therapeutically-effective amount of an antisense oligonucleotide with MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES or I-309 interactive capability to produce therapeutic effects on areas of potential restenosis.

24. An antisense oligonucleotide with MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES or I-309 interactive capability to therapeutically inhibit vascular restenosis upon administration to a warm-blooded animal.

25. The antisense oligonucleotide with MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES or I-309 interactive capability of claims 23, 24, or 25 wherein said peptide labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate is capable of

administration to a warm-blooded animal to produce therapeutic effects on areas of potential restenosis.

26. A composition comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 or a combination of two or more thereof or derivatives thereof which retains MCP-1 interactive capability conjugated with a N_3S ligand having the general structure

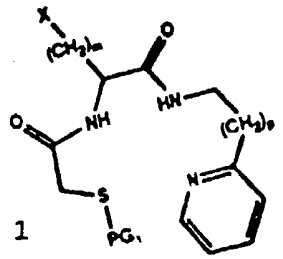

Figure 1

wherein m is a whole number less than eleven; p is either 0 or 1; PG_1 is a sulfur protecting group selected from the group consisting of C_{1-20} S-acyl, C_{1-20} alkyl, C_{1-10} alkoxyalkyl, carbamoyl and C_{1-10} alkoxycarbonyl and X is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl.

27. A composition comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 or an antisense molecule having MCP-1 interactive capability or a combination of two or more thereof or derivatives thereof conjugated with a N_2S_2 ligand having the general structure

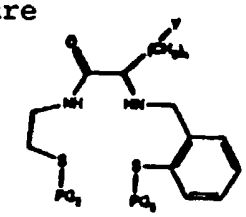

Figure 2

wherein n is a whole number less than eleven; PG_2 and PG_3 may be the same or different sulfur protecting groups selected from the group consisting of C_{1-20} S-acyl, C_{1-20} alkyl, C_{1-10} alkoxyalkyl, carbamoyl and C_{1-10} alkoxycarbonyl and Y is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl.

28. A composition comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 or an antisense molecule having MCP-1 interactive capability or a combination of two or more thereof or derivatives thereof conjugated with a phenolic ligand having the general structure

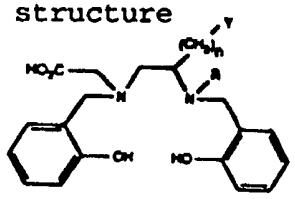

Figure 3

wherein n is a whole number less than eleven; Y is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; and R is hydrogen or a C_{1-10} alkyl.

29. A composition comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 or an antisense molecule having MCP-1 interactive capability or a combination of two or more thereof or derivatives thereof conjugated with a metal complex having the general structure

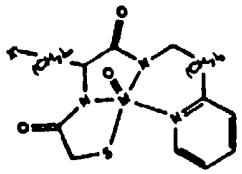

Figure 4

wherein m is a whole number less than eleven; p is either 0 or 1; X' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

30. A composition comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 or an antisense molecule having MCP-1 interactive capability or a combination of two or more thereof or derivative thereof conjugated with a metal complex having the general structure

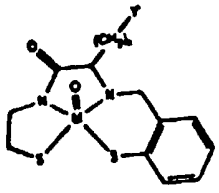

Figure 5

wherein Y' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; n is a whole number less than eleven; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

31. A composition comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 or an antisense molecule having MCP-1 interactive capability or a combination of two or more thereof or derivatives thereof conjugated with a metal complex having the general structure

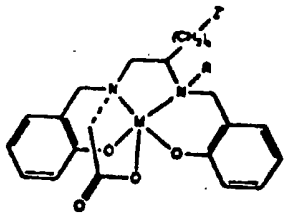

Figure 6

wherein q is a whole number less than eleven; wherein Z' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; R is selected from the group consisting of hydrogen, and C_{1-10} alkyl; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

32. A composition comprising the oligonucleotide of claim 1, antisense MIP-1 Alpha, MIP-1 Beta, RANTES precursor, RANTES, I-309 or an antisense molecule having MCP-1 interactive capability or a combination of two or more thereof or derivatives thereof conjugated with a metal complex having the general structure

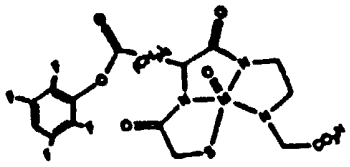

Figure 7

wherein M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

33. The composition of claim 26 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

34. The composition of claim 27 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium

gluconate or tartarate.

35. The composition of claim 28 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

36. The composition of claim 29 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

37. The composition of claim 30 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

38. The composition of claim 31 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

39. The composition of claim 32 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

40. The composition of claim 26 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

41. The composition of claim 27 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

42. The composition of claim 28 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

43. The composition of claim 29 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

44. The composition of claim 30 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

45. The composition of claim 31 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

46. The composition of claim 32 labelled with ^{111}In-indium derivatives such as indium chloride, or tartarate.

47. The composition of claim 26 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

48. The composition of claim 27 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

49. The composition of claim 28 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

50. The composition of claim 29 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

51. The composition of claim 30 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium

gluconate or tartarate.

52. The composition of claim 31 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

53. The composition of claim 32 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

54. The composition of claim 26 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

55. The composition of claim 27 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

56. The composition of claim 28 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

57. The composition of claim 29 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

58. The composition of claim 30 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

59. The composition of claim 31 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

60. The composition of claim 32 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

61. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an

effective amount of the composition of claim 33 for diagnostic imaging of areas of potential restenosis.

62. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 34 for diagnostic imaging of areas of potential restenosis.

63. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 35 for diagnostic imaging of areas of potential restenosis.

64. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 36 for diagnostic imaging of areas of potential restenosis.

65. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 37 for diagnostic imaging of areas of potential restenosis.

66. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 38 for diagnostic imaging of areas of potential restenosis.

67. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 39 for diagnostic imaging of areas of potential restenosis.

68. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an

effective amount of the composition of claim 40 to produce therapeutic effects on areas of potential restenosis.

69. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 41 to produce therapeutic effects on areas of potential restenosis.

70. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 42 to produce therapeutic effects on areas of potential restenosis.

71. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 43 to produce therapeutic effects on areas of potential restenosis.

72. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 44 to produce therapeutic effects on areas of potential restenosis.

73. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 45 to produce therapeutic effects on areas of potential restenosis.

74. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 46 to produce therapeutic effects on areas of potential restenosis.

75. The composition of claim 33, wherein M is ^{99m}technetium.

76. The composition of claim 34, wherein M is ^{99m}technetium.

77. The composition of claim 35, wherein M is ^{99m}technetium.

78. The composition of claim 36, wherein M is ^{99m}technetium.

79 The composition of claim 37, wherein M is ^{99m}Technetium.

80. The composition of claim 38 wherein M is ^{99m}Technetium.

81. The composition of claim 39 wherein M is ^{99m}Technetium.

82. The composition of claim 40, wherein M is indium-111.

83. The composition of claim 41, wherein M is indium-111.

84. The composition of claim 42, wherein M is indium-111.

85. The composition of claim 43, wherein M is indium-111.

86. The composition of claim 44, wherein M is rhenium-186 or rhenium-188.

87. The composition of claim 45, wherein M is rhenium-186 or rhenium-188.

88. The composition of claim 46, wherein M is rhenium-186 or rhenium-188.

89. The composition of claim 47, wherein M is rhenium-186 or rhenium-188.

90. The composition of claim 48, wherein M is rhenium-186 or rhenium-188.

91. The composition of claim 49 wherein M is rhenium-186 or rhenium-188.

92. The composition of claim 50, wherein M is rhenium-186 or rhenium-188.

93. The composition of claim 51, wherein M is rhenium-186 or rhenium-188.

94. The composition of claim 52, wherein M is rhenium-186 or rhenium-188.

95. The composition of claim 53 wherein M is rhenium-186 or rhenium-188.

96. The composition of claim 54, wherein M is yttrium-90.

97. The composition of claim 55 wherein M is yttrium-90.

98. The composition of claim 56, wherein M is yttrium-90.

99. The composition of claim 57, wherein M is yttrium-90.

100. The composition of claim 58, wherein M is yttrium-90.

101. The composition of claim 59, wherein M is yttrium-90.

102. The composition of claim 60, wherein M is yttrium-90.

103. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 61 to image areas of potential restenosis.

104. A method of performing a diagnostic procedure, which

comprises administering to a warm-blooded animal an effective amount of the composition of claim 62 to image areas of potential restenosis.

105. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 63 to image areas of potential restenosis.

106. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 64 to image areas of potential restenosis.

107. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 65 to image areas of potential restenosis.

108. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 66 to image areas of potential restenosis.

109. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 67 to image areas of potential restenosis.

110. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 68 to image areas of potential restenosis.

111. A method of performing a diagnostic procedure, which

comprises administering to a warm-blooded animal an effective amount of the composition of claim 69 to image areas of potential restenosis.

112. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 70 to image areas of potential restenosis.

113. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 71 to image areas of potential restenosis.

114. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 72 to image areas of potential restenosis.

115. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 73 to image areas of potential restenosis.

116. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 74 to image areas of potential restenosis.

117. A composition suitable for administration to a warm blooded animal comprising an antisense MCP-1 peptide or a derivative thereof to inhibit vascular restenosis.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/00605

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 514/2, 6, 44, 492; 536/24.5; 424/1.69, 1.73; 436/504
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 6, 44, 492; 536/24.5; 424/1.69, 1.73; 436/504

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	European Heart Journal, Volume 12, (Abstract Supplement), issued 1991, M. Ebbecke et al., "Inhibition of Human Arterial Smooth Muscle Cell Proliferation by a c-myc Antisense Oligonucleotide", page 11, abstract no. 177, see entire abstract.	1-117
Y	FEBS Letters, Volume 244, No. 2, issued February 1989, T. Yoshimura et al, "Human Monocyte Chemoattractant Protein-1 (MCP-1), Full-Length cDNA Cloning, Expression in Mitogen-Stimulated Blood Mononuclear Leukocytes, and Sequence Similarity to Mouse Competence Gene JE", pages 487-493, see entire document.	1-117

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 MARCH 1995	Date of mailing of the international search report 20 APR 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHARLES C. P. RORIES Telephone No. (703) 308-0196

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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemical and Biophysical Research Communications, Volume 147, No. 1, issued 31 August 1987, D. Mercola et al., "Antisense RNA to the c-fos Gene: Restoration of Density-Dependent Growth Arrest In a Transformed Cell Line", pages 288-294, see entire document.	6
Y	The FASEB Journal, Volume 5, No. 5, issued 15 March 1991, E. Wickstrom et al., "Antisense DNA Methylphosphonate Inhibition of c-*myc* Gene Expression in Transgenic Mice", page A1443, abstract no. 6218, see entire abstract.	1-117
Y	Biochemical and Biophysical Research Communications, Volume 149, No. 3, issued 31 December 1987, U. Rasmussen et al., "On Antisense Peptides: The Parathyroid Hormone as an Experimental Example and a Critical Theoretical View", pages 930-938, see entire document.	7-10, 12, 15-117
Y	Proceedings of the National Academy of Sciences USA, Volume 88, issued June 1991, S. Yla-Herttuala et al., "Expression of Monocyte Chemoattractant Protein 1 in Macrophage-rich Areas of Human and Rabbit Atherosclerotic Lesions", pages 5252-5256, see entire document.	1-117
Y	Journal of Clinical Investigation, Volume 88, issued October 1991, N. Nelken et al., "Monocyte Chemoattractant Protein-1 in Human Atheromatous Plaques", pages 1121-1127, see entire document.	1-117
Y	Journal of Nuclear Medicine, Volume 32, No. 3, issued March 1991, S. Butler et al., "Rapid Localization of Indium-111-Labeled Inhibited Recombinant Tissue Plasminogen Activator in a Rabbit Thrombosis Model", pages 461-467, see entire document.	11-117
Y	US, A, 4,965,392 (FRITZBERG ET AL.) 23 October 1990, columns 1-10.	11-117
Y	US, A, 5,037,630 (FRITZBERG ET AL.) 06 August 1991, columns 1-25.	11-117
Y	US, A, 4,707,440 (STAVRIANOPOULOS) 17 November 1987, columns 1-10.	11-117

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/00605

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Cancer Research, Volume 50, issued 15 December 1990, M. Goldrosen et al., "Biodistribution, Pharmacokinetic, and Imaging Studies with ^{186}Re-labeled NR-LU-10 Whole Antibody in LS174T Colonic Tumor-bearing Mice", pages 7973-7978, see entire document.	11-117
Y	Nuclear Medicine and Biology, Volume 18, No. 5, issued 1991, S. Schwarz et al., "Evaluation of Two New Bifunctional Chelates for Radiolabeling a Parathyroid-specific Monoclonal Antibody with In-111", pages 477-481, see entire document.	11-117
Y	Inorganic Chemistry, Volume 29, No. 16, issued 1990, N. Bryson et al., "Protecting Groups in the Preparation of Thiolate Complexes of Technetium", pages 2948-2951, see entire document.	11-117

INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.